Translational Readthrough of the Murine Leukemia Virus *gag* Gene Amber Codon Does Not Require Virus-Induced Alteration of tRNA

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An in vitro system to assay translational readthrough of the UAG termination codon at the murine leukemia virus (MuLV) *gag-pol* junction was developed by using rabbit reticulocyte lysates programmed by SP6-generated Moloney MuLV *gag-pol* mRNA. Under conditions in which the suppressor activity of the lysate was dependent on addition of tRNA, it could be shown that readthrough synthesis was stimulated to approximately the same extent by equivalent amounts of tRNA from MuLV-infected and uninfected NIH 3T3 cells. Analysis of glutamine tRNA, which mediates suppression in vivo, showed that the level of glutamine acceptor activity and the chromatographic profile of glutamine isoacceptors were unchanged following virus infection. On the basis of these results, we conclude that the suppressor tRNA occurs normally within the tRNA population of uninfected cells and need not be induced in response to virus infection.

The *pol* proteins of murine leukemia virus (MuLV) are positioned on the genetic map in the order 5'-protease-reverse transcriptase-IN (integration protein)-3' (3, 17, 19, 22, 33) and are made from a large 200-kilodalton (kDa) precursor polypeptide, Pr200^{*gag-pol*}, which is cleaved at a late stage in virus assembly (4). The amount of Pr200^{*gag-pol*} produced in MuLV-infected cells is only 1/10 to 1/50 of that of the *gag* precursor protein Pr65^{*gag*} (16). This observation led to the suggestion that synthesis of the MuLV *gag-pol* precursor is subject to translational control (16). On the basis of the ability of a *Saccharomyces cerevisiae* amber suppressor tRNA to stimulate Pr200^{*gag-pol*} synthesis in rabbit reticulocyte lysates programmed by virion RNA (25, 28), Philipson et al. postulated that expression of Pr200^{*gag-pol*} during virus infection is regulated by readthrough suppression of a UAG termination codon at the 3' boundary of the *gag* gene (28). The existence of a UAG codon separating the *gag* and *pol* coding regions was subsequently confirmed by sequence analysis of the Moloney (Mo) (31) and AKR (7, 11) MuLV genomes. Determination of the N-terminal amino acid sequence of the purified MuLV protease protein demonstrated that the UAG *gag* codon is read as glutamine in the protease, suggesting that suppression of the amber codon is mediated by a glutamine tRNA (33). Further evidence for the involvement of glutamine tRNA was provided by the finding that starvation of MuLV-infected cells for glutamine, but not for other essential amino acids such as tyrosine and leucine, inhibits synthesis of Pr200^{*gag-pol*} and blocks proteolytic processing of Pr65^{*gag*} (8). Recently, Kuchino et al. reported that MuLV-infected cells contain elevated levels of a minor glutamine tRNA which was able to suppress the UAG codon of tobacco mosaic virus RNA in vitro (20).

The goal of this study was to investigate readthrough suppression and the role of glutamine tRNA in MuLV infection. An in vitro suppression assay was developed by using a defined Mo-MuLV *gag-pol* fragment as the template for synthesis of viral mRNA. Our results demonstrate that tRNA from normal cells has the same suppressor activity as

tRNA isolated from MuLV-infected cells. Furthermore, we found that virus infection has no effect on either the level of glutamine tRNA acceptor activity or the distribution of glutamine isoacceptor species.

To perform this work, it was necessary to devise a system which would allow us to detect synthesis of MuLV *gag-pol* fusion proteins. The in vitro assay that was developed is similar in experimental design to assays described earlier (14, 28). A Mo-MuLV fragment containing sequences extending from the *Xba*I site in U3 (nucleotide -151 [31]) to the *Hind*III site in *pol* (nucleotide 4894 [31]) was inserted into an SP6 expression vector, and the resulting clone was designated as pMXH (see the schematic diagram in Fig. 1). The cloned DNA was transcribed into viral mRNA, which was used to program a rabbit reticulocyte lysate (Fig. 1). In the absence of mRNA (lane 1), immunoprecipitation with anti-p30 revealed no protein bands. Addition of mRNA generated from *Sal*I-digested pMXH (lane 2), however, resulted in the synthesis of three major products which could be detected by immunoprecipitation with anti-p30: Pr65^{*gag*}; a 75-kDa protein, presumably the *gag* protein which is glycosylated in vivo (4); and a 115-kDa protein having the size predicted for a *gag-pol* protein encoded by sequences extending to the *Sal*I site. As noted in other studies with reticulocyte lysates (13, 25, 28), there were also several additional virus-specific bands which could result from (i) proteolytic cleavage by the viral protease or cellular proteases in the lysate, (ii) internal initiation of protein synthesis, (iii) premature termination of translation, or (iv) a combination of these factors. Direct visualization of the ³⁵S-labeled protein products gave a pattern identical to that obtained after immunoprecipitation with anti-p30, except for an additional band corresponding to hemoglobin which was synthesized both with and without viral mRNA (data not shown). No viral proteins were detected by immunoprecipitation with normal serum (lane 3).

Further evidence that a *gag-pol* fusion protein is one of the products synthesized in the reticulocyte lysate was obtained in experiments with mRNA transcribed from *Hind*III-digested pMXH DNA. Immunoprecipitation of the proteins

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FIG. 1. Synthesis of Mo-MuLV *gag* and *gag-pol* proteins in a rabbit reticulocyte lysate. Clone pMXH was constructed as follows. A 5-kilobase *Xba*I-*Hind*III fragment from clone pMR1, derived from an infectious clone of Mo-MuLV (originally obtained from David Steffen [29]) and containing sequences extending from an *Eco*RI site in 5'-flanking DNA to a unique *Hind*III site in *pol*, was isolated and inserted between the *Xba*I and *Hind*III sites of the expression vector pGem4Z (Promega Biotec) downstream of the SP6 promoter. Viral mRNA was generated from clone pMXH following digestion with either *Sal*I (lanes 2 and 3) or *Hind*III (lanes 4 and 5) by using SP6 RNA polymerase under the conditions specified by Promega Biotec and was used to program viral protein synthesis in a rabbit reticulocyte lysate. Protein synthesis was performed in 50- μ l reactions containing 20 μ M hemin, 80 mM potassium acetate, 0.5 mM magnesium acetate, 19 amino acids (minus methionine) at 20 μ M each, 50 μ g of creatine phosphokinase per ml, 10 mM creatine phosphate, 1 mM dithiothreitol, 1 μ l of RNasin (specific activity, 40 U/ μ l), 1 μ g of mRNA, 30 μ Ci of [35 S]methionine (specific activity, 1,134 Ci/mmol; New England Nuclear Corp.), and 31 μ l of lysate supplemented with 50 μ g of calf liver tRNA (Promega Biotec) per ml. The reactions were incubated for 60 min at 30°C. [35 S]methionine-labeled viral proteins were immunoprecipitated with the antiserum indicated below each lane as previously described (12) and then subjected to electrophoresis in a sodium dodecyl sulfate-8% polyacrylamide gel. Anti-p30 serum (α p30) was obtained from the National Cancer Institute (lot 79S-117). The designation normal refers to normal rabbit serum. Anti-reverse transcriptase (α RT) serum was anti-pRT24, which contains antibodies to the AKR MuLV *pol* fusion protein expressed by clone pRT24 (12). No mRNA was added to the reaction analyzed in lane 1. The molecular size markers shown on the left were myosin (200K), phosphorylase *b* (97.4K), bovine serum albumin (69K), and human γ -globulin (53K). A schematic diagram of the Mo-MuLV insert in clone pMXH and the upstream SP6 promoter region is shown above the gel. The positions of the restriction sites, the AUG initiation codon, and the UAG termination codon separating the *gag* and *pol* coding regions are indicated; the relative distance between the SP6 and MuLV sequences is not drawn to scale.

made in response to this mRNA with anti-p30 (lane 4) also revealed the presence of three major products: the two *gag* proteins and a 157-kDa protein. The difference in size between the 115- and 157-kDa proteins corresponds to the expected difference between the molecular masses of the translated nucleotide sequences extending to the *Sal*I (nu-

cleotide 3705 [31]) and *Hind*III sites. As anticipated, anti-MuLV reverse transcriptase serum (12) also reacted with the 157-kDa protein but not with the *gag* proteins (lane 5). The 82- and 91-kDa bands, which appeared to be more prominent after immunoprecipitation with anti-reverse transcriptase (compare lanes 4 and 5), may have resulted from internal initiation of protein synthesis followed by premature termination of translation (13). The molar ratio of the 115- and 157-kDa *gag-pol* fusion proteins to Pr65^{gag} , as determined by densitometry measurements (and taking the differential methionine content into account), was approximately 1:50 (lanes 2 and 4). This value is consistent with the relative amounts of $\text{Pr200}^{\text{gag-pol}}$ and Pr65^{gag} found in MuLV-infected cells (16). Similar results were obtained when 35S viral RNA was used to program rabbit reticulocyte lysates (25, 28).

The results shown in Fig. 1 demonstrate that the UAG termination codon at the *gag-pol* junction is suppressed in the *in vitro* system and that the efficiency of translation of *pol* sequences is similar to that observed *in vivo*. Thus, the *in vitro* system can be used to assay tRNA suppressor activity. To investigate the abilities of various tRNAs to stimulate *gag-pol* synthesis, we used a rabbit reticulocyte lysate which was not supplemented with exogenous tRNA; activity was tested with mRNA generated from *Hind*III-digested pMXH DNA (Fig. 2). In the absence of exogenous tRNA, the major viral proteins synthesized were Pr65^{gag} and the 75-kDa *gag* protein, whereas only trace amounts of the 157-kDa protein were detectable (Fig. 2A, lane 3, and B, lane 1). In contrast, unfractionated tRNA from uninfected (Fig. 2A, lane 1) and Mo-MuLV-infected (Fig. 2A, lane 2) NIH 3T3 cells each stimulated synthesis of the *gag-pol* fusion protein. Both tRNA samples, which were added in equal amounts, had equivalent suppressor activities. The same level of suppressor activity was also observed with an equal amount of unfractionated rabbit liver tRNA (data not shown).

To determine whether tRNA from a nonmammalian source can suppress the MuLV amber termination codon, we tested the activities of yeast tRNAs (a generous gift from Ray Gesteland) in the unsupplemented reticulocyte lysate (Fig. 2B). Addition of unfractionated tRNA from a yeast strain carrying a serine amber suppressor (lane 2) stimulated synthesis of the 157-kDa *gag-pol* protein, whereas an equivalent amount of tRNA from a control wild-type strain had a small inhibitory effect on the synthesis of *gag* proteins and showed no suppressor activity (lane 3). Synthesis of the *gag-pol* protein was also stimulated by a partially purified yeast tyrosine amber suppressor tRNA (lane 4). These results are in accord with previous reports (25, 28) and demonstrate that the ability to suppress the MuLV UAG codon is not restricted to a unique tRNA species in either uninfected or infected mouse cells.

To further investigate the finding that tRNA preparations from MuLV-infected and uninfected NIH 3T3 cells show the same amount of suppressor activity (Fig. 2A), we varied the concentration of tRNA added to reactions performed with the unsupplemented reticulocyte lysate and then determined the effect on *gag-pol* protein synthesis (Fig. 3). In these experiments, viral mRNA was transcribed from *Sal*I-digested pMXH DNA. Analysis of reactions with tRNA from either uninfected (lanes 1 to 4) or infected (lanes 5 to 8) cells showed that the amount of the 115-kDa readthrough product synthesized was dependent on the amount of tRNA added. With low concentrations of tRNA (2 to 4 μ g/ml; lanes 1, 2, 5, and 6), there was little or no synthesis of the 115-kDa protein; as the concentration of tRNA was increased to 8

FIG. 2. Stimulation of Mo-MuLV *gag-pol* protein synthesis by tRNA from Mo-MuLV-infected and uninfected NIH 3T3 cells and yeast tRNAs. Viral protein synthesis directed by viral mRNA transcribed from *HindIII*-digested pMXH DNA was performed in a rabbit reticulocyte lysate treated for 6 (A) or 8 (B) min with micrococcal nuclease (15) and prepared without supplementation with tRNA. Conditions for the protein synthesis reactions, immunoprecipitation of viral proteins with anti-p30, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis are given in the legend to Fig. 1. To obtain tRNA from infected and uninfected cells, duplicate cultures of NIH 3T3 cells were transfected with the infectious Mo-MuLV clone (see the legend to Fig. 1) or carrier calf thymus DNA alone. The cells were passaged in parallel in Dulbecco modified Eagle medium containing 10% fetal bovine serum (GIBCO Laboratories), and the fluid was changed 1 day before they reached confluence. On the next day, fluid samples were taken for tests of infectivity (1) and reverse transcriptase activity (21). (Typically, control samples had <2 focus-inducing units/ml and incorporated <1 pmol of [³H]TMP per ml in response to poly(rA)-oligo(dT), whereas MuLV samples had approximately 10⁶ focus-inducing units/ml and incorporated approximately 300 pmol of [³H]TMP per ml). The cells were removed from the flasks by treatment with 0.05% trypsin-0.53 mM EDTA (GIBCO) or by being scraped with a sterile rubber policeman into Tris-buffered saline, and they were then harvested by low-speed centrifugation at 1,000 × *g*. tRNA was isolated from cells by extraction with phenol and chloroform and then applied to a DE-52 cellulose column (0.5 by 2 cm) as previously described (2). The yeast tRNAs were the generous gift of Ray Gesteland, Howard Hughes Medical Institute, University of Utah Medical Center, Salt Lake City. The tRNAs added to the protein synthesis reactions were from the following sources: (A) Lanes: 1, uninfected NIH 3T3 cells (40 μg/ml); 2, Mo-MuLV-infected NIH 3T3 cells (40 μg/ml); 3, no addition. (B) Lanes: 1, no addition; 2, unfractionated yeast amber suppressor tRNA (160 μg/ml); 3, unfractionated yeast nonsuppressor tRNA (160 μg/ml); 4, partially purified yeast tyrosine suppressor tRNA (20 μg/ml). The molecular size markers shown on the left were the same as those in Fig. 1.

μg/ml (lanes 3 and 7) and 20 μg/ml (lanes 4 and 8), a corresponding increase in this protein could be seen. Furthermore, at each tRNA concentration tested, the suppressor activities of samples from infected and uninfected cells were similar (the activity of tRNA from infected cells was about two- or threefold lower than that of samples from uninfected cells). These results demonstrate that suppressor activity is not enriched in Mo-MuLV-infected cells.

The findings of Fig. 2A and 3 also imply that virus infection does not affect the cellular tRNA population. To investigate this possibility, we acylated tRNA preparations from infected and uninfected cells with eight different amino acids (Table 1). The data showed no significant differences between the specific acceptor activities of matched samples. It is of particular interest that glutamine acceptor activity (Table 1, line 2) was the same in infected and uninfected cells.

Analysis of isoacceptor species of individual tRNAs was performed by fractionation on a reverse-phase chromato-

FIG. 3. Effect of varying the concentration of tRNA from Mo-MuLV-infected and uninfected NIH 3T3 cells on Mo-MuLV *gag-pol* protein synthesis. The experimental procedures were as described in the legend to Fig. 2A, except that viral mRNA was transcribed from *Sall*-digested pMXH DNA and the reticulocyte lysate was treated with micrococcal nuclease for 8 min. Reactions were supplemented with tRNA from uninfected (lanes 1 to 4) or Mo-MuLV-infected (lanes 5 to 8) NIH 3T3 cells. The concentrations of tRNA added were as follows. Lanes: 1 and 5, 2 μg/ml; 2 and 6, 4 μg/ml; 3 and 7, 8 μg/ml; 4 and 8, 20 μg/ml.

graphic column (RPC-5) (18) as previously described (9, 10). The tRNA profiles obtained for asparagine, aspartic acid, histidine, phenylalanine, tyrosine, and valine isoacceptors were unaffected by MuLV infection of NIH 3T3 cells (data not shown). With glutamine, a double-label experiment was performed so that samples from infected and uninfected cells (matched with respect to passage number) could be cochromatographed on the same RPC-5 column (Fig. 4). Two major isoacceptor peaks and one minor isoacceptor peak were resolved, and no differences between the relative proportions of these isoacceptors were detected. Analysis of AKR MuLV-infected and uninfected SC-1 cells (23) gave glutamine tRNA profiles very similar to those illustrated in Fig. 4 (data not shown), indicating that our present findings are not restricted to Mo-MuLV-infected and uninfected NIH 3T3 cells. Varying the ATP and Mg²⁺ concentrations in the acylation mixture over a wide range (e.g., 6 and 20 mM,

TABLE 1. Aminoacylation of tRNA from MuLV-infected and uninfected NIH 3T3 cells

Amino acid ^a	pmol/A ₂₆₀ U of tRNA ^b	
	MuLV-infected cells	Uninfected cells
Alanine	32.1	31.1
Glutamine	23.1	28.8
Glycine	63.0	71.5
Histidine	21.5	16.1
Leucine	35.7	33.5
Lysine	61.0	58.4
Proline	38.1	35.1
Tyrosine	13.9	12.7

^a ¹⁴C-labeled amino acids were purchased from Amersham Corp. and had the following specific activities (mCi/mmol): alanine, 354; glutamine, 288; glycine, 114; histidine, 604; leucine, 623; lysine, 604; proline, 285; tyrosine, 531.

^b Amino acid acceptance was determined by measuring the attachment of ¹⁴C-labeled amino acids under conditions in which the tRNA concentration was limiting. The appropriate tRNA concentration was determined independently for each acylation reaction. Reactions (final volume, 25 μl) containing 25 mM Tris hydrochloride (pH 7.4), 10 mM potassium acetate, 20 mM magnesium acetate, 6 mM ATP, 0.25 μCi of ¹⁴C-labeled amino acid, 19 unlabeled amino acids (minus the radioactive amino acid) at 0.2 mM each, 5 to 50 μg of tRNA, and 1.5 μl of an aminoacyl-tRNA synthetase preparation from rabbit reticulocytes (9, 10) were incubated for 15 min at 37°C and then assayed for trichloroacetic acid-insoluble radioactivity.

FIG. 4. RPC-5 column chromatography of glutamine tRNA from Mo-MuLV-infected and uninfected NIH 3T3 cells. tRNA ($0.2 A_{260}$ U) from Mo-MuLV-infected NIH 3T3 cells was acylated with $25 \mu\text{Ci}$ of $[^3\text{H}]$ glutamine (50 Ci/mmol) in a $250\text{-}\mu\text{l}$ reaction, and tRNA ($1 A_{260}$ U) from uninfected NIH 3T3 cells was acylated with $3.8 \mu\text{Ci}$ of $[^{14}\text{C}]$ glutamine (285 mCi/mmol) in a $500\text{-}\mu\text{l}$ reaction, as described in Table 1, footnote *b*, except that proportionately higher amounts of the aminoacyl-tRNA synthetase preparation were used. Incubation was for 20 min at 37°C . After phenol extraction and ethanol precipitation, the ^3H - and ^{14}C -labeled tRNAs were cochromatographed on an RPC-5 column (18) as detailed elsewhere (9, 10). Two-milliliter fractions were collected and counted directly in Aquasol.

respectively [Fig. 4]; 8 and 10 mM, respectively [Fig. 5]; or 2 and 3 mM, respectively, which are the conditions used by Kuchino et al. [20] had little or no effect on the profiles that were obtained (data not shown). The results indicate that virus infection has neither a qualitative nor a quantitative effect on glutamine tRNA species.

To further characterize the glutamine tRNA from Mo-MuLV-infected NIH 3T3 cells and to verify that the fractionation of individual isoacceptors was consistent with the normal pattern (9), we examined the coding properties of each tRNA peak (Fig. 5). In this run, four peaks were resolved: two major peaks, I and III, and two minor peaks, II and IV. Fractions from each peak were pooled, as indicated by the cross-hatching, and assayed by the ribosomal filter-binding assay of Nirenberg and Leder (26). As shown at the top of Fig. 5, peaks I and II, representing most of the total glutamine tRNA acceptor activity, responded to the glutamine code word CAG. Peak III recognized CAA, whereas peak IV responded to CAG and CAA, showing a slight preference for CAG. None of the tRNA pools showed any response to the termination codon UAA or UAG. These results are in accord with previous studies on bovine and rabbit liver tRNAs (9) and indicate that the chromatographic profiles and coding properties of glutamine tRNA from MuLV-infected cells are essentially the same as those of glutamine tRNA from normal mammalian tissues. The lack of response to either of the termination codons is not surprising; for example, both functional tyrosine suppressor tRNA having guanine in the first position of the anticodon and the corresponding inactive suppressor having the highly modified base queuine in place of the guanine are unable to recognize UAG in the ribosomal binding assay (30; D. L. Hatfield, unpublished data).

From the data presented in this paper, we conclude that translational readthrough of the amber codon at the 3'

terminus of *gag* is mediated by a tRNA which is normally present in uninfected cells and need not be induced in response to virus infection. This conclusion, which is in accord with an earlier suggestion by Philipson et al. (28), is based on our finding that synthesis of MuLV *gag-pol* proteins is stimulated to approximately the same extent by tRNA from MuLV-infected and uninfected cells (Fig. 2 and 3). Moreover, we have shown in other studies that MuLV *gag-pol* proteins are also made in translation reactions directed by SP6-generated viral mRNAs carrying either an ochre (UAA) codon or an opal (UGA) codon in place of the UAG termination signal; in these cases too, equivalent suppressor activity by tRNA from infected and uninfected cells is observed (Y.-X. Feng, J. G. Levin, D. L. Hatfield, T. S. Schaefer, R. J. Gorelick, and A. Rein, *J. Virol.*, in press). Our results complement the recent findings of Panganiban (27), who reported that the amber codon in a plasmid containing a 300-base-pair MuLV *gag-pol* junction fragment fused in frame to the *Escherichia coli lacZ* gene was suppressed at a frequency of 10%, as evidenced by expression of β -galactosidase, when the plasmid was transfected into various normal vertebrate cells. Significantly, the amount of fusion protein detected, either by reaction with a monoclonal antibody or by enzymatic activity, was not increased in MuLV-infected NIH 3T3 cells (27).

Further support for the notion that a normally occurring tRNA is involved in MuLV suppression is derived from our data demonstrating that the level of glutamine acceptor activity (Table 1) and distribution of isoacceptor species (Fig. 4) are not affected by virus infection. These results are in contrast to those of Kuchino et al. (20). This group has reported that a minor glutamine tRNA which recognizes CAA and suppresses the tobacco mosaic virus RNA amber codon in vitro is present at an extremely low level in uninfected NIH 3T3 cells but is increased more than 10-fold

FIG. 5. Codon responses of glutamine isoacceptor tRNAs from Mo-MuLV-infected NIH 3T3 cells. Ten A_{260} U of tRNA isolated from Mo-MuLV-infected NIH 3T3 cells (Fig. 2, legend) was acylated with 0.5 mCi of [3 H]glutamine in a 2.5-ml reaction as described in Table 1, footnote *b*, except that the ATP and Mg^{2+} concentrations were 8 and 10 mM, respectively, 0.15 ml of the aminoacyl-tRNA synthetase preparation was used, and the reaction was incubated for 20 min. After phenol extraction and ethanol precipitation, the [3 H]-labeled glutamine tRNA (5.9×10^6 cpm) was applied to an RPC-5 column (Fig. 3, legend). Two-milliliter fractions were collected, and 20- μ l portions were counted directly in Aquasol. Fractions from four regions (I to IV) were pooled, as indicated by the cross-hatching, and precipitated with ethanol. The precipitated tRNAs were collected on 25-mm-diameter nitrocellulose filters (pore size, 0.45 μ m) and eluted with 150 μ l of water. Portions of each pool (6 μ l of peaks I and III and 15 μ l of peaks II and IV) were used to measure codon-specific binding to *Escherichia coli* ribosomes by the procedure of Nirenberg and Leder (26), as previously described (9). The tabulated results are shown. Codons are listed in the first column to the left. For each tRNA fraction, radioactivity bound to ribosomes in the absence of the added codon (None) is given in parentheses and was subtracted in each case from the values obtained in the presence of the codon. The corrected values (Δ cpm) are the specific amounts of radioactivity bound to ribosomes in response to the indicated codons.

following MuLV infection. This augmented level of CAA-decoding glutamine tRNA (20) is similar to the level which we normally observe in both infected and uninfected cells (Fig. 4 and 5) and also in mammalian liver (9). Profound stimulation of the synthesis of a glutamine tRNA isoacceptor should be reflected in concomitant stimulation of total glutamine acceptor activity, but in fact, examination of this activity revealed no increase in infected cells (Table 1). At present, the reasons for the difference between our results and those of Kuchino et al. (20) are not clear. More importantly, however, the functional data on suppression of termination codons in vitro (Fig. 2 and 3; Feng et al., in press) and in vivo (27) emphasize that production of the MuLV *gag-pol* precursor is not dependent on elevation of the suppressor tRNA in infected cells or on virus-induced modification of this tRNA. Presumably, other factors e.g., codon context or downstream stem-and-loop structures (5, 6, 24, 27, 32; Feng et al., in press) do have a critical influence. Experiments designed to analyze the signals required for translational readthrough are in progress.

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